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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 09/965,101 | 09/26/2001 | Arthur M. Krieg | C01039/70057/MAT | 3959 |

7590 04/10/2003

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| EXAMINER |
|---|
| NGUYEN, DAVE TRONG |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1632 | 9 |

DATE MAILED: 04/10/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

# Office Action Summary

| Application No. | Applicant(s) |
|---|---|
| 09/965,101 | Krieg |
| **Examiner** | **Art Unit** |
| Dave Nguyen | 1632 |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1) ☒ Responsive to communication(s) filed on *Jan 21, 2003*.

2a) ☐ This action is **FINAL.** 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

**Disposition of Claims**

4) ☒ Claim(s) *59-108* is/are pending in the application.

4a) Of the above, claim(s) *71, 77, 83, 88, 101, 105, and 108* is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) *59-70, 72-76, 78-82, 84-87, 89-100, 102-104, 106, and 107* is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9) ☐ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on *Sep 26, 2001* is/are a) ☒ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. §§ 119 and 120**

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). _____
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

Applicant's election with traverse of a species of DNA motif that is GTCGTT, a species of promoter that is a viral promoter, and a species of therapeutic polypeptide that is a growth factor. As the result of the orally modified restriction indicated to applicants on January 3, 2003, the group restriction has been vacated by the examiner, and as such, applicant's election in the response filed January 21, 2003 is fully responsive to the modified restriction. In addition, the elected claimed invention has been withdrawn by the examiner, and thus, the species restriction also has been withdrawn by the examiner.

Claims 59-108 are pending for examination.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 59-108 are rejected under 35 U.S.C. 112, first paragraph, because the specification is enabling only for claims limited to:

1/ A method for enhancing the expression of a polypeptide in a mammalian or avian subject, wherein the polypeptide is encoded by a nucleic acid contained in a nucleic acid construct comprising:

(a) determining the presence of immunostimulatory unmethylated CpG motifs (CpG-S motifs) in non-essential nucleotide regions of a nucleic acid construct encoding a polypeptide;

(b) removing the CpG-S motifs present in the non-essential nucleotide regions of the nucleic acid construct; and/or

(c) inserting neutralizing CpG (CpG-N) motifs into a nucleotide region of the construct, wherein the region comprises non-essential nucleotide residues and CpG-S motifs;

(d) administering the construct of step (b) and/or (c) to a mammalian or avian subject;

thereby enhancing the expression of the polypeptide in the subject.

The specification does not reasonably provide enablement for a method of enhancing the expression of any therapeutic polypeptide *in vivo* by simply performing the steps as claimed in the base claim 59 and/or 84. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

With respect to the preamble of the base claims and claims dependent therefrom, the presently pending claims encompass a method of making immunstimulatory DNA coding for a therapeutic polypeptide comprising the step of inserting a CpG-N motif into any region of a DNA construct encoding a therapeutic polypeptide, wherein the region does not necessarily comprise at least on CpG-S motif. The application essentially teaches the followings:

- The CpG-N motifs are CpG motifs containing an unmethylated CpG dinucleotide, which are actually immunostimulatory on their own under the proper experimental conditions.
- The CpG-N motifs only exhibit an immune-neutralizing effect when the motifs are present in a DNA construct comprising CpG-S motifs.

Thus, it is apparent that the application coupled with state of the prior art as a whole only teaches the making of immunostimulatory nucleic acid constructs in the context of having at least one CpG-S motif (as defined by the application and claims). However, the claims as presently pending are readable on the making of nucleic acid constructs that contain an insertion of any CpG-N motif into any region of nucleotide residues which do not necessarily contain any CpG-S motif. More specifically and to further support the immunostimulatory activities of CpG-N motifs, Table I of Branda *et al.* summarized works done in the prior art, indicates that sequences containing tetranuleotides CCGC do stimulate B-cell proliferation rather than effecting an immune-neutralizing and/or immune-inhibitory effects (items 16, 30, and 31, for example). Thus, it is not apparent as to how one skilled in the art, without any undue experimentation, makes and use the claimed nucleic acid constructs which must exhibit an enhanced expression of any therapeutic polypeptide contained therein, especially when the active step of inserting CpG-N motif into a nucleic acid construct alone not only does not necessarily contribute to an enhanced expression of any therapeutic polypeptide, but also effects an immunostimulating response, which in turns decreases the expression of the polypeptides by the nucleic acid constructs *in vivo*, particularly in view of the teachings from the as-filed specification and the state of the prior art.

Moreover and with respect to presently pending claims embracing the active steps of removing CpGS and/or inserting CpG-N in any region of the nucleic acid construct including regions containing essential nucleotide residues, *e.g.,* essential and/or required for the expression and/or desired activity of the therapeutic polypeptide, the as-filed specification does not provide any sufficient guidance as to how to employ the claimed steps without effecting the desired activities and/or structures of the essential nucleotide residues contained in the nucleic acid construct.

Notwithstanding the issues as set forth above, and with respect to claimed embodiments encompassing therapeutic applications of DNA constructs including viral vector that comprises a DNA coding for a therapeutic polypeptide and/or antisense sequence to treat a disease or disorder *in vivo*, it is not apparent as to how one skilled in the art, without any undue experimentation, use any nucleic acid construct other than nucleic acid constructs coding for an antigen as therapeutic nucleic acid molecules to generate any and/or therapeutic response against any disease or disorder, especially within the context of the guidance and evidence provided by the as-filed specification. The specification only provides sufficient guidance and/or factual evidence demonstrating an increase in humoral immune response and/or cytotoxic lymphocyte (CTL) response by injection into the tibialis anterior (TA) muscle of mice a hepatitis antigen (HBs-Ag) encoded plasmid construct, wherein the plasmid construct also comprises immunostimulatory-unmethylated CpG(S) motifs (Table 3) as compared to the plasmid construct without the CpG(S) motifs, *e.g.,* Figure 9.

However, major considerations for any gene transfer or any DNA therapy protocol involve issues that include:

1/ The type of vector and amount of DNA constructs to be administered;

2/ The route and time course of administration, the sites of administration, the trafficking of the genetic material within cellular organelles, the rate of degradation of the DNA, the level of mRNA produced, the stability of the mRNA product, the amount and stability of the protein produced, and

3/ What amount of the expressed proteins considered to be therapeutically effective for a DNA therapy method (Anderson, Nature, Vol. 392, pp. 25-30, April 1998).

In addition, all of these issues differ dramatically based on the specific vector used, the route of administration, the animal being treated, therapeutically effective amount of the DNA, and the disease being treated. Riddell *et al.* (Nature Medicine, Vol. 2, 2:216-223, 1996) teaches that even with the use of autologous

cells expressing a foreign protein, wherein the autologous are supposedly non-immunogenic, HIV-infected patients when grafted with autologous cytotoxic CD8+ T cells induce strong primary T-cell immune responses to foreign antigens expressed by transferred autologous cytotoxic CD8+ T cells (p. 221, column 1), and that the rejection of genetically modified cells by immunocompromised hosts suggests that strategies to render gene-modified cells less susceptible to host immune surveillance will be required for successful gene therapy of immuno-competent hosts (abstract, page 221, column 1). As such, it is not apparent how a simple reduction of an immune response against the nucleic acid construct but not the expressed polypeptide can be reasonably extrapolate to the make and use of any nucleic acid construct as claimed within the context of therapeutic applications.

More specifically, the state of the art exemplified by Verma, Nature, Vol. 389, pages 239-242, 1997, states that "the Achilles heel of gene therapy is gene delivery", that "thus far, the problem has been an inability to deliver genes efficiently and to obtain sustained expression", that gene delivery methods using non-viral vectors "suffer from poor efficiency of delivery and transient expression of the gene", and that "although there are reagents that increase the efficiency of delivery, transient expression of the transgene is a conceptual hurdle that needs to be addresses" (page 239, column 3, first paragraph). In addition, Anderson summarized the state of the art before 1998, and teaches that gene therapy is a powerful new technology that still requires several years before it will make a noticeable impact on the treatment of disease, and that several major deficiencies still exist including poor delivery systems, both viral and non-viral, and poor gene expression after genes are delivered (page 30, column 1, last paragraph). Anderson further teaches that the reason for the low efficiency of gene transfer and expression in human patients is that we still lack a basis understanding of how vectors should be constructed, what regulatory sequences are appropriated for which cell types (page 30, column 1, last paragraph). Furthermore, Verma *et al.* indicate that factors including the nature of the diseases and/or disorders, the nature of a DNA and/or target tissue, and a delivery system and/or amounts of the DNA complexes employed in the delivery system that would generate a therapeutic effect *in vivo* must be considered for any gene therapy method to be successful (page 238, columns 1 and 2). The specification does not provide sufficient guidance and/or factual evidence demonstrating a reasonable correlation between the disclosure including its exemplified examples and the subject matter being sought in the claims wherein any DNA construct (plasmid and viral vectors) containing CpG-N motifs and/or containing no CpG-S motif can be used as

therapeutic molecule against any disease or disorder, particularly given all of the reasons set forth above. Thus, it is not apparent how one skilled in the art determines, without undue experimentation, which of the disclosed DNA complexes as broadly claimed in the claimed methods generate therapeutic effect in any and/or all nucleic acid therapy methods as contemplated by the as-filed specification, particularly given the unpredictability of nucleic acid therapy as a whole and/or the doubts expressed in the art of record.

Even if the presently pending claims encompass a method of employing a nucleic acid plasmid construct coding for any therapeutic polypeptide to generate a therapeutic effect in any and/or all subjects including any and/or all animals, *e.g.,* reptiles, birds, mammals, amphibians, wherein any administration route including non-injection routes, *e.g.,* inhalation or oral route is contemplated, the application only provides sufficient guidance and/or factual evidence demonstrating an increase in humoral immune response and/or cytotoxic lymphocyte (CTL) response by injection into the tibialis anterior (TA) muscle of mice a hepatitis antigen (HBs-Ag) encoded plasmid construct, wherein the plasmid construct also comprises immunostimulatory-unmethylated CpG(S) motifs (Table 3) as compared to the plasmid construct without the CpG(S) motifs, *e.g.,* Figure 9.

The application and claims are not enabling for methods of using any and/or all claimed DNA as therapeutic molecules which are employed by any and/or all administration routes to provide a protection and/or a therapeutic effect against any and/or all diseases in any and/or all animals (referred as '*in vivo*' in the claimed methods) at the time the invention was made. The application contemplates that by employing any DNA construct as claimed in any *in vivo* environment, a therapeutic effect elicited by the administered DNA can be generated regardless of the numerous huddles expressed by the art of the record that must be overcomed. While the application provides sufficient guidance and/or factual evidence showing an increased immune response in mice wherein intramuscular injection of a composition comprising a HBs-Ag) encoded plasmid construct comprising immunostimulatory-unmethylated CpG(S) motifs is employed, the application does not provide sufficient guidance and/or any factual evidence to demonstrate a protective effect and/or therapeutic effect by using any other claimed DNA constructs commensurate with the intended scope of the claims. In addition to the doubts expressed in Anderson and Verma, the state of the art exemplified by Plenat (J. Mol. Med. Today, Vol.2, No.6:250-257, 1996) states that "Oligonucleotides do not seek out their specific targets...The observed biological effects *in vivo* are transient; this has implications if a long-term effect is desired", that "the magnitude and nature of the responses are critical factor" (p. 256, last paragraph). In addition, Plenat states:

Approximately 16 clinical trials are currently in progress. However, relatively little is known about the *in vivo* behaviour of oliogonucleotides. Extrapolations from *in vitro* studies to predict *in vivo* pharmacokinetics and effects in humans might be difficult and inappropriate (abstract).

The route of administration and the dose are both of major importance in the development of oligonucleotides as therapeutic agents. Maintaining sufficient local concentrations of the intact, biologically active drugs is essential to obtain the desired biological effects. In animals, oligonucleotides have been administered by the intravenous (i.v.), intraperotneal (i.p.), subscutaneous (s.c.)...Previous pharmacokinetic studies demonstrated that intravenously administered nanoparticles are largely trapped by the liver, especially by the Kupffer cells, and this limits the bioavailability of drugs carried by them (page 252, column 2, first paragraph).

Even though modified olionucleotides are now being used, the inhibition of gene expression using antisense oligonucleotides is inefficient, and high doses are most often necessary to achieve significant results...The doses of phosphorothioate oligonucleotide administered to animals for pharmacokinetic studies are highly variable (1-130 mg kg –1), which greatly complicates the interpretations of results (page 252, column 2, second paragraph);

Even with modified ODNs, Stull *et al.,* Pharmaceutical Res., Vol. 12, pp. 465-483, 1995) states problems associated with specific activity and delivery routes remain problematic in the field of antisense therapy:

Chemical modifications to nucleic acid polymers have significantly improved the stability of nucleic acid drugs, although these modifications may adveserly affect the affinity or activity of the reagent (page 476, column 1, third paragraph).

It is clear, however, that application of these expensive compounds [apatamers, antigene oligonucleotides and ribozymes] *in vivo* requires many problems be solved; some of which have been discussed above in conjunction with studies performed in cell culture, but particularly those related to delivery *in vivo* of nucleic acids to the cytoplasm of specific cells. The delivery challenge can be subdivided into problems with persistence of effect, access to the target cells and efficient cytoplasmic delivery of the drug (page 476, column 2, last paragraph).

In summary advances in molecular biology and synthetic chemistry have led to novel nucleic acid drugs to inhibit gene expression and protein function. However, the delivery and entry of nucleic acid drugs into the target site remains a major obstacle to the successful introduction of this aspect of the molecular biology revolution into a clinical setting (page 478, column 1, last paragraph).

Thus, it is not apparent as to how one skilled in the art reasonably extrapolates, without undue experimentation, from the disclosure of the application to the full scope of the claimed invention that would generate a protective effect and/or a therapeutic effect in any or all subjects against any disease or disorder. Even if a protective and/or therapeutic response has been shown in mice using the exemplified mice, it is not apparent as to how the mouse model wherein intramuscular injection of one single species of a HBs-Ag) encoded plasmid is reasonably extrapolated to the full scope of the claimed invention, particularly given that there is no evidence that the mice model is a general phenomenon, and given the doubts expressed in the art of record.

With respect to claimed methods encompassing any route of administration wherein the only intended use of an *in vivo* expression of a therapeutic polypeptide expressing construct is to produce therapeutically relevant effect *in vivo,* the state of the art exemplified by Meng *et al.* (Gene Therapy of Cancer, Chapter I, pp. 3-20, 1999) teaches that factors including specific genes used for a treatment, gene delivery vectors, routes of administration, and gene expression are all critical for the success of a gene therapy method (pages 4-6). For example, Meng *et al.* teach that "it is difficult to prepare sufficiently high titers of retroviruses for *in vivo* gene therapy", that "the most significant drawback to adenoviruses, however, is that they elicit a strong host immune

response", and that "although it may seem intuitive that a heightened immune response may be good in cancer gene therapy, it is less desirable on a practical scale because the immune response helps to eliminate the vector and to decrease the expression of the transduced gene" (p. 4, column 2, last paragraph). Meng *et al.* further teach that "although animal studies have suggested low toxicity and excellent efficacy, these investigations have been limited by the use of immuno-deficient mice" (p. 6, column 1).

With respect to administration routes, Meng *et al.* teach that other than intratumor injection, delivery of virally expressed genes by intravascular or intracavitary injections also presents barriers to the delivery of the target genes (p. 6, column 1). For example, Meng *et al.* state:

> "In intravascular administration, instillation into a peripheral vein dilutes the vehicle, so only a small portion may ultimately reach the tumor. Intravascular administration also elicits a powerful immune response. Tropism for organs such as the liver, for example by adenovirus, can be a disadvantage if delivery is intended elsewhere or may be advantageous if the liver is the target. Even with regional intravascular administration, the virus must traverse the endothelial wall and travel against pressures within an expanding tumor mass. In the case of intracavitary administration (i.e., intrapleural or intraperitoneal), the surface of the tumor mass is coated by virus, but intratumoral delivery within a solid mass represents an important barrier" (page 6, column 1).

At best, the as-filed specification coupled with the state of the prior art at the time the invention was made only provides sufficient guidance and/or evidence to reasonably enable the claimed invention directed to an improvement in delivering and expressing a polypeptide in a mammal or avian subject by making and use the nucleic acid constructs with the provisions as set forth on pages 2 and 3 of this office action.

Thus, it is not apparent how one skilled in the art, without undue experimentation, practices the full scope of the claimed invention, particularly on the basis of applicant's disclosure, and in view of the doubts expressed in the art of record at the time the invention was made.

Claims 62 and 96 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Claim 62 and claim 96 are dependent from the base claim 59 and 84, respectively, which clearly are limited to nucleic acid constructs comprising a nucleic acid encoding a polypeptide. Since the nucleic acid constructs of the base claims encodes and expresses a therapeutic

polypeptide, the nucleic acid constructs of the base claims, particularly on the basis of the application disclosure, must be necessarily expression vectors. Thus, the recitation of a functional limitation "expression vector" fails to further limit the subject matter of a previous claim Applicant is required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form.

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner *Dave Nguyen* whose telephone number is **(703) 305-2024**.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, *Deborah Reynolds*, may be reached at **(703) 305-4051.**

Any inquiry of a general nature or relating to the status of this application should be directed to the *Group receptionist* whose telephone number is **(703) 308-0196**.

Dave Nguyen
Patent Examiner
Art Unit: 1632

DAVE T. NGUYEN
PRIMARY EXAMINER